Results and analysis of performance in proficiency test 34 and 35

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EURL-*Campylobacter* Workshop 2023

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Thank you for your participation and for providing information in the questback reports!

Number of participants

Year	2023	2022	2021	2020	2019	2018	2017	2016	2015	2014
	PT 34	PT 31	PT 29	PT 26	PT 23	PT 21	PT 19	PT 17	PT 15	PT 13
Enumeration	35	34	33	33	35	37	36	36	36	35
	PT 35	PT 32	PT 30	PT 27	PT 24	PT 22	PT 20	PT 18	PT 16	PT 14
Detection & species id	32	31	36	29	33	31	34	33	32	36

PT 34: Enumeration (and species identification)

Proficiency test No. 34

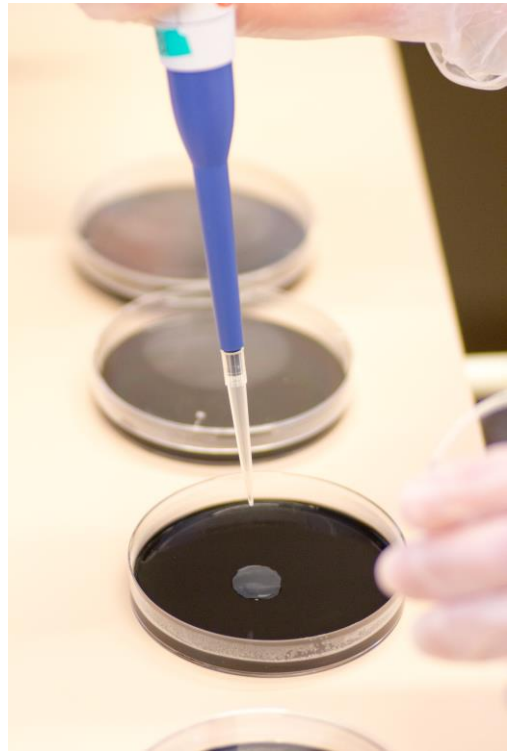
Objective: to assess the performance of the NRLs to enumerate (and voluntary species identify) *Campylobacter* in chicken skin

- Enumeration and confirmation of *Campylobacter* spp. in chicken skin
- Species identification of *Campylobacter* (voluntary)
- Recommended method ISO 10272-2:2017, but other methods allowed
- Should allow enumeration of between 10 and 10^5 cfu *Campylobacter*/g chicken skin

PT 34: Contents and procedure

- One bag of about 120 g chicken skin to be divided into 10 portions of 10 g
- 10 vials with freeze-dried sample (with or without *Campylobacter*)
- Make an initial dilution of 10^{-1} and homogenise
- Follow the method(s) of choice for
  - enumeration
  - species identification (voluntary)

  } of *Campylobacter* spp.



PT 34: Description of the 10 vials

Sample No.	Species	Level (log cfu/vial)		Batch No.
1	*C. jejuni* & *Escherichia coli*	4.19	3.56	SLV313
2	*C. lari*	4.86		SLV335
3	*C. coli*	6.67		SLV374
4	*C. coli*	5.36		SLV333
5	Negative			
6	*C. lari*	4.86		SLV335
7	*C. coli*	6.67		SLV374
8	*Escherichia coli*		4.29	SVA079
9	*C. jejuni*	3.81		SLV306
10	*C. jejuni* & *Escherichia coli*	4.19	3.56	SLV313

PT 34: Quality control

- Vials produced and tested for homogeneity and stability by the Swedish Food Agency / EURL
- Before selection for the PT, the EURL did enumeration of two or three vials per batch together with chicken skin to ensure levels and functionality
- The EURL performed the complete test the day after dispatch
- The EURL did additional enumerations on vials with *Campylobacter* to test stability during transport conditions

Test of stability during transport conditions

Test occasion	Storage condition	No. of samples tested
Before dispatch	Best case	Each vial with *Campylobacter* × 2
Two days after dispatch	Best case	The complete test
Two weeks after dispatch	Worst case	Each vial with *Campylobacter* × 3

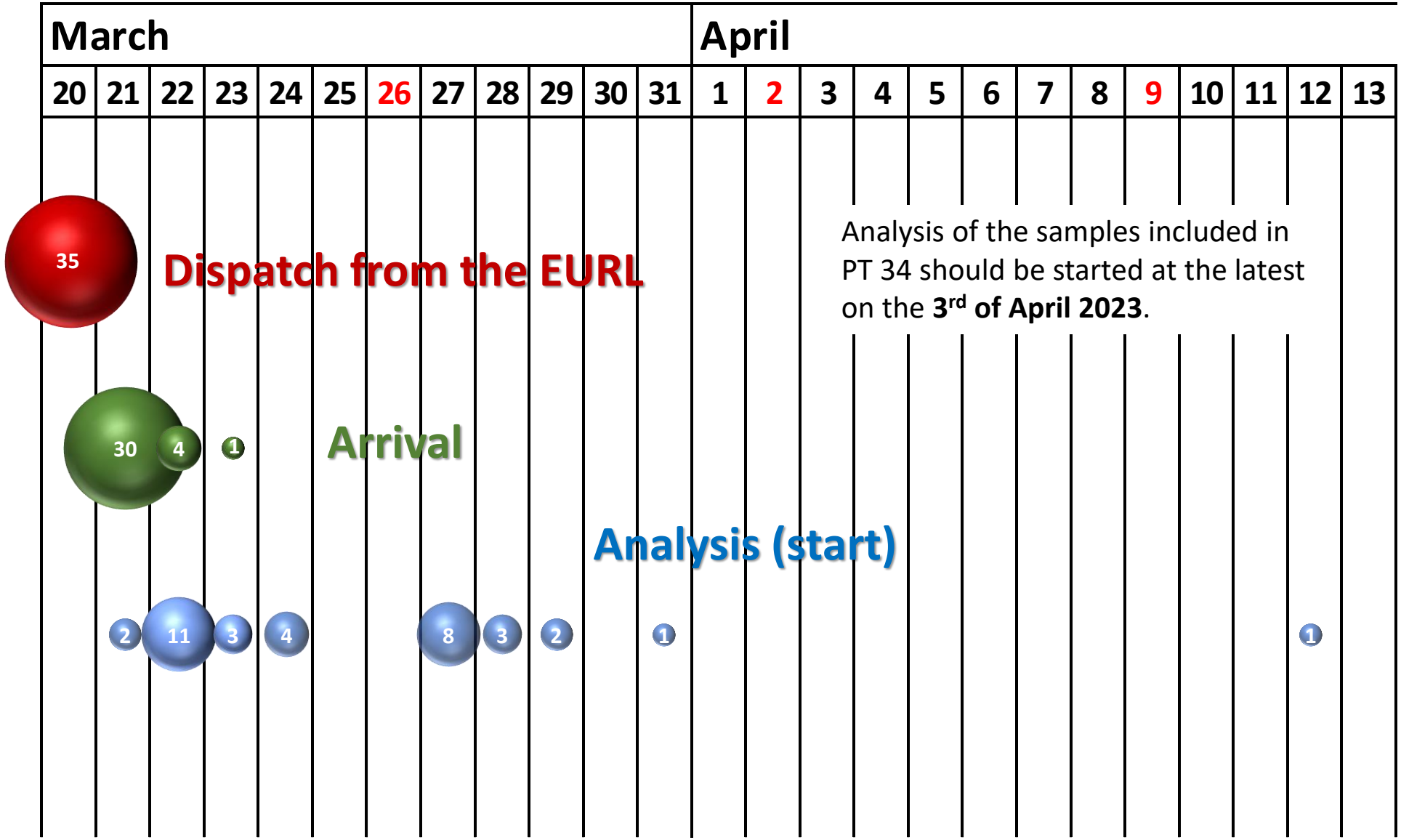
Best case: 5 °C for 24 h

Worst case: 5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h

PT 34: Preparation of the chicken skin

- Chicken thigh skin delivered from a slaughterhouse with low level of *Campylobacter*-positive flocks and a farm with no positive flocks for more than 1 year
- Tested in triplicates with enrichment in Bolton and Preston broth, as well as direct streak on mCCD and Preston agar
- All samples tested negative for presence of *Campylobacter* but moderately with background flora was present
- Cut into pieces and divided into portions of about 120 g
- Stored at −20 °C until distribution

PT 34: Time to arrival & start of analysis



PT 34: How was performance calculated?

- The Median Absolute Deviation (MADe) to calculate performance
- σMADe = MADe × 1.4826
- *Campylobacter*-containing samples
  - Results within participants' median ±2σMADe = 2 points
  - Results between ±2σMADe and ±3σMADe = 1 point
  - Results outside ±3σMADe = 0 points
- *Campylobacter*-negative samples
  - No *Campylobacter* reported = 2 points
  - False positive result = 0 points
- The maximum score (2 points for each sample) was 20 points
- Calculate the score for each participant

Grade	Scoring limits	
Excellent	20	95.1–100%
Good	17–19	85.0–95.0%
Acceptable	14–16	70.0–84.9%
Needs improvement	12–13	57.0–69.9%
Poor	<12	<57.0%

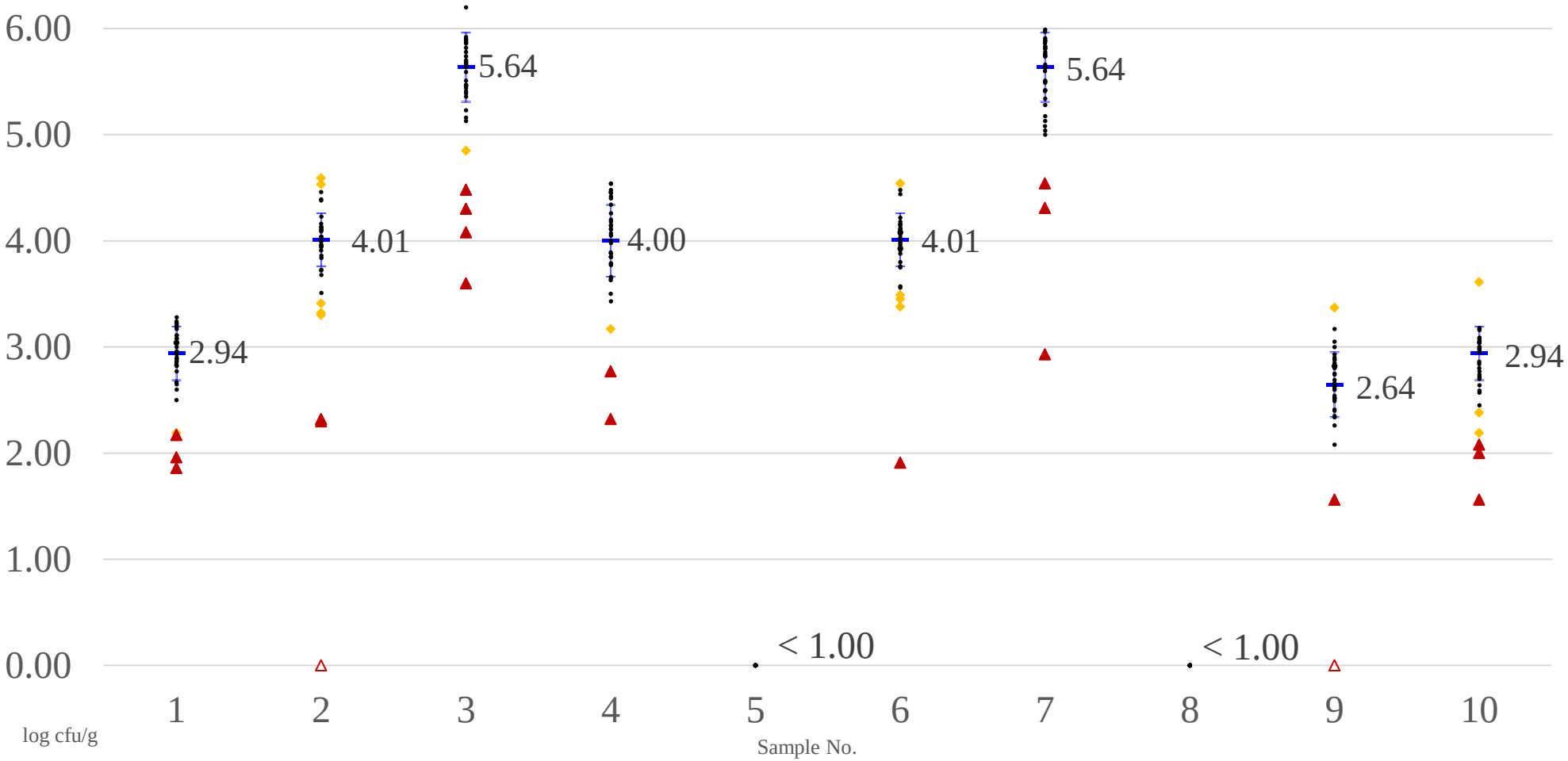
PT 34: How was performance calculated?

Adaptions because of homogenous results and use of duplicates

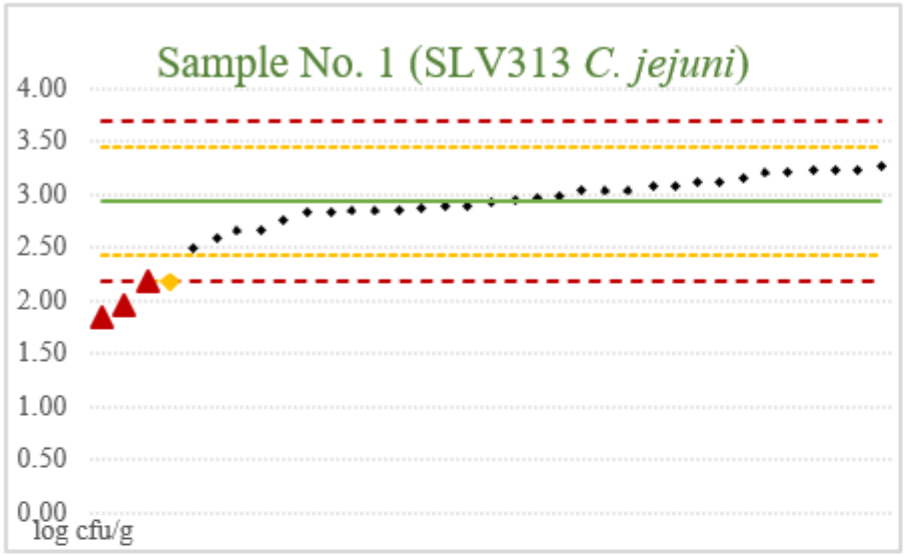
- **Homogeneous results** (2 and 6)
  - σMADe adjusted to 0.25 $\log_{10}$ cfu/g, according to the 0.5 $\log_{10}$ rule (ISO 22117:2019)
- **Duplicate vials** (1 and 10, 2 and 6, and 3 and 7)
  - Median and σMADe calculated for 1) each single sample, 2) each pair of samples
  - For performance evaluation: duplicate values used,

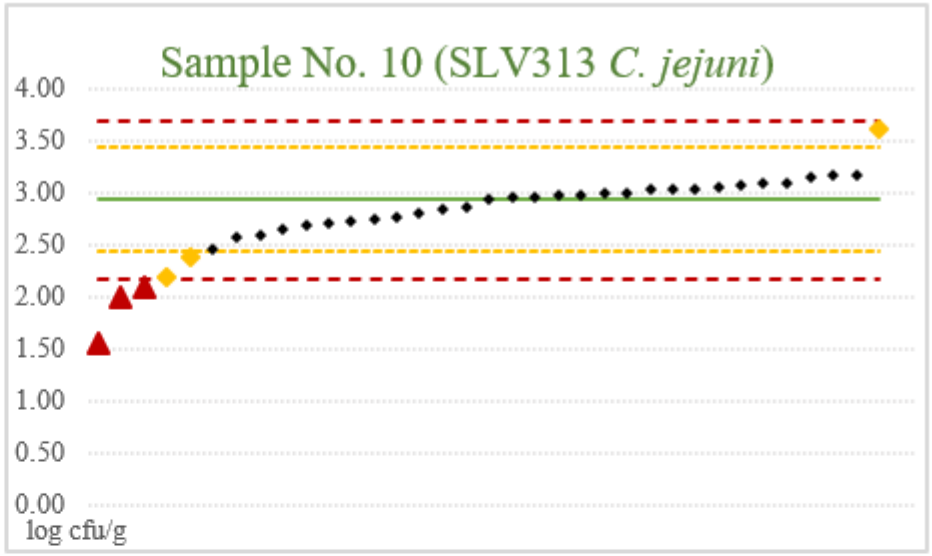
    thus the same scoring limits applicated for both samples in a pair
- No sample in PT 34 had a −3σMADe limit below 1.0 $\log_{10}$ cfu/g
  - No adjustment of the minimum score for negative results

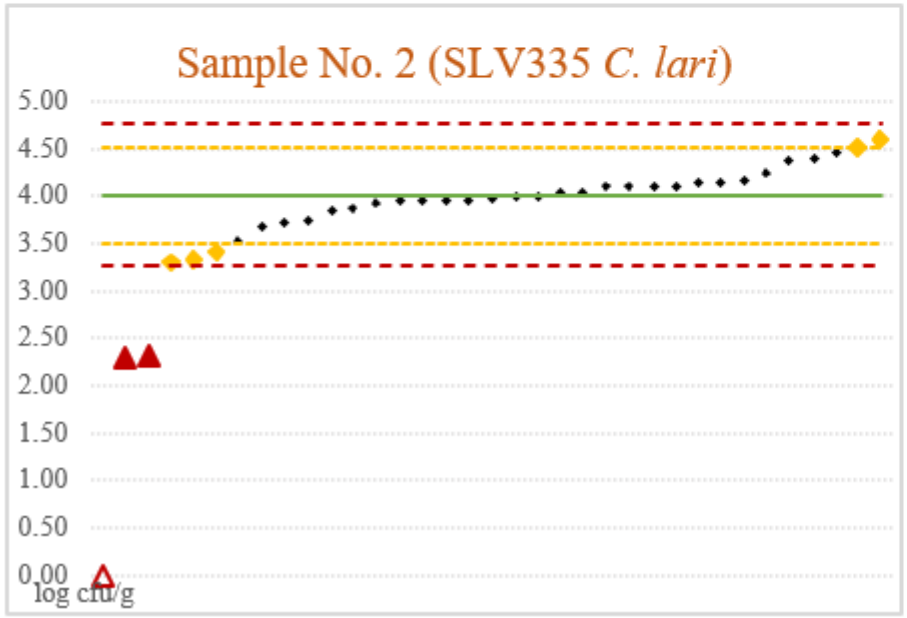
PT 34: Results of enumeration

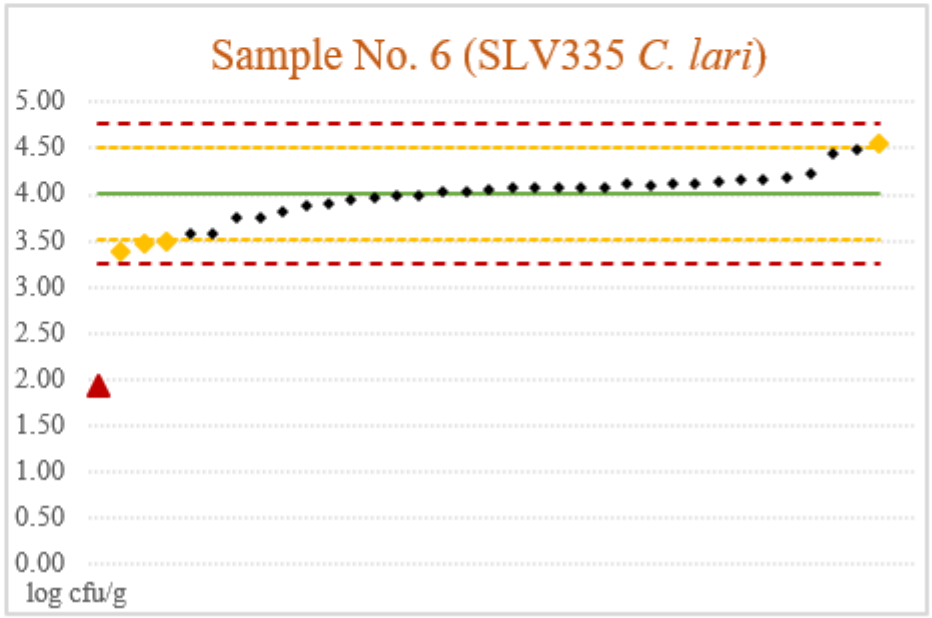


PT 34: Results of enumeration

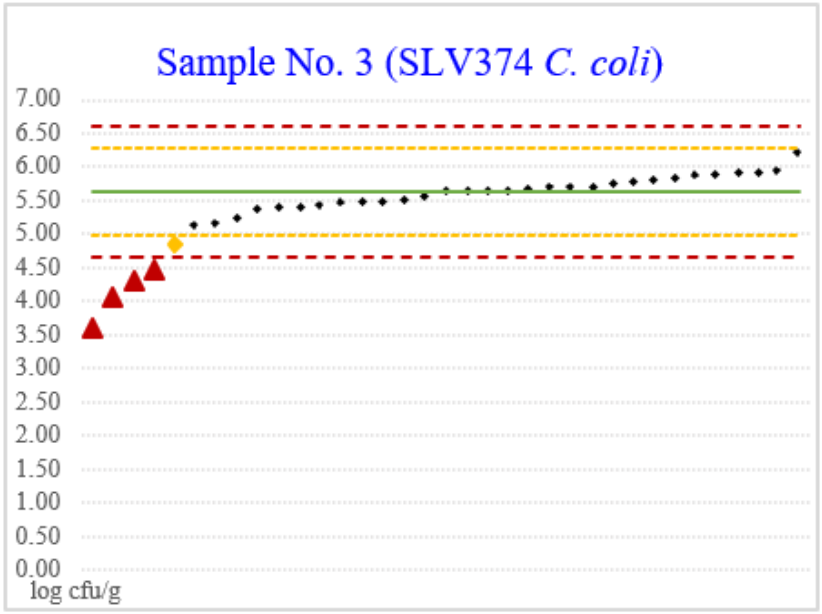


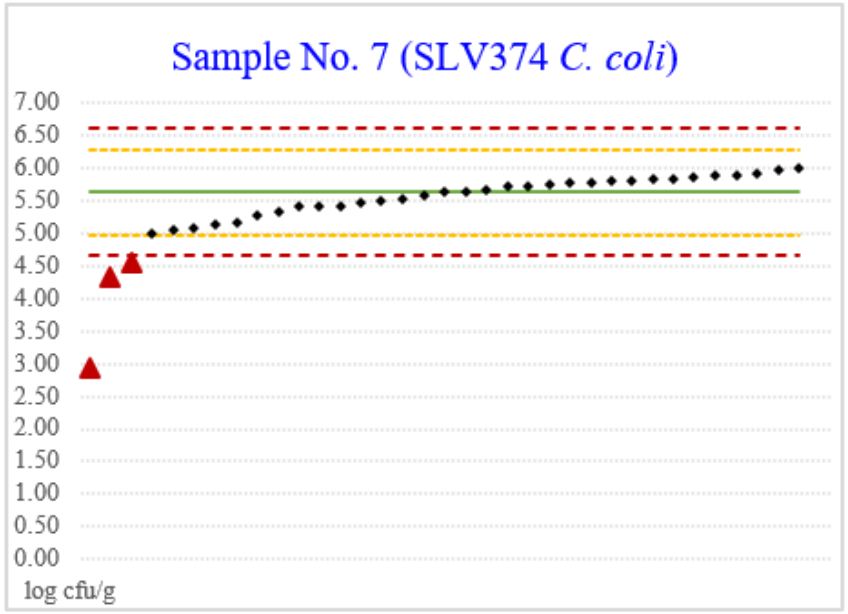


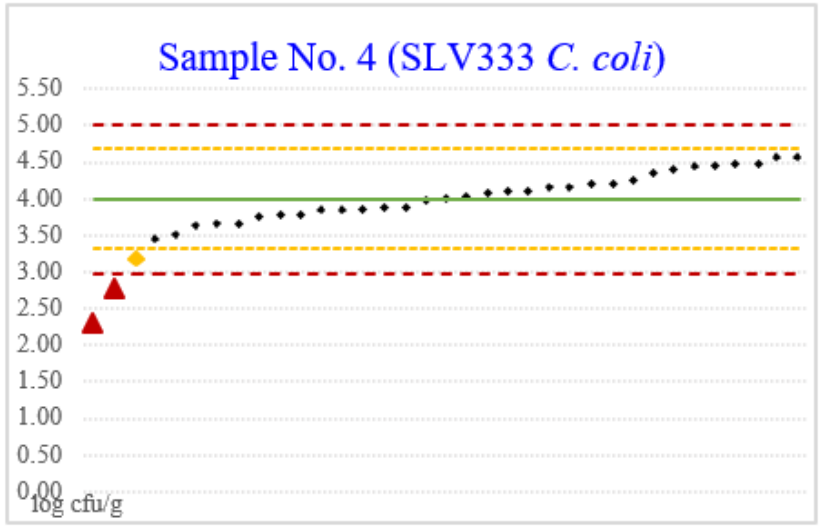


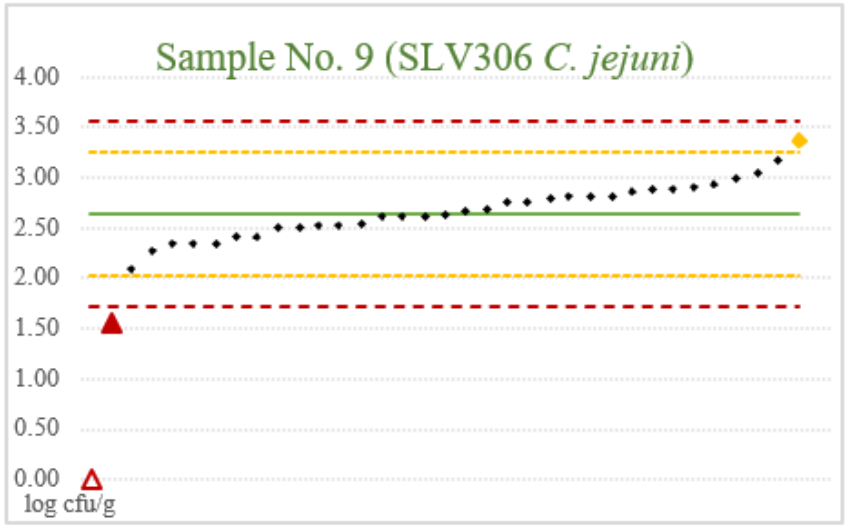


PT 34: Results of enumeration





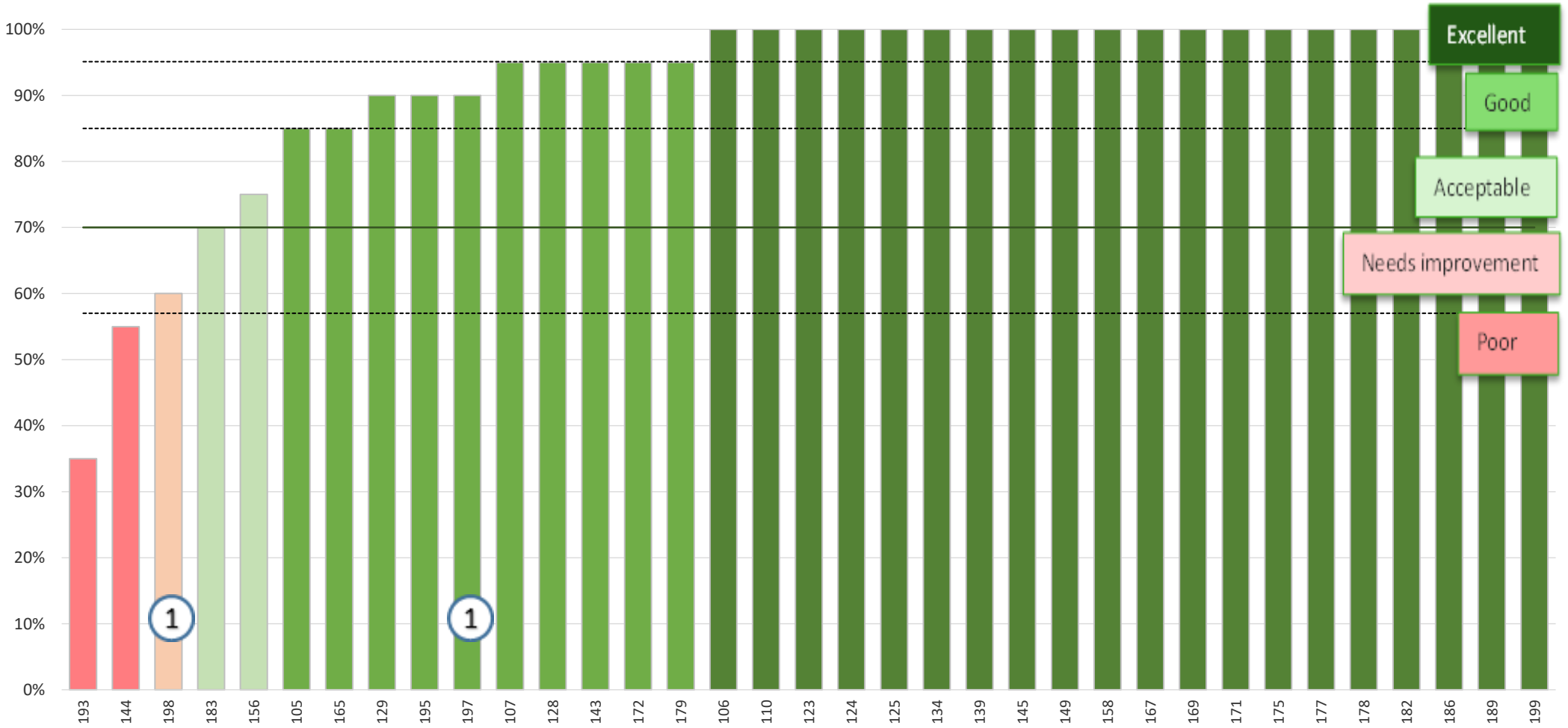




Variability in PT enumeration results

		max-min diff (between labs)				MADe in PT			
Year	*PT*	*max*	*min*	*mean*	*median*	*max*	*min*	*mean*	*median*
2017	**19**	5.90	2.19	**3.54**	3.23	0.37	0.23	**0.30**	0.29
2018	**21**	4.06	1.80	**3.02**	3.31	0.49	0.17	**0.30**	0.28
2019	**23**	2.48	1.27	**1.88**	1.94	0.24	0.19	**0.21**	0.22
2020	**26**	3.36	0.92	**1.89**	1.75	0.32	0.13	**0.24**	0.24
2021	**29**	2.65	1.89	**2.17**	2.08	0.45	0.29	**0.37**	0.38
2022	**31**	3.50	0.96	**1.94**	1.92	0.31	0.12	**0.18**	0.16
2023	**34**	3.59	1.42	**2.60**	2.62	0.23	0.10	**0.18**	0.19
	mean	3.65	1.49	2.43	2.39	0.34	0.18	0.25	0.25

PT 34: Performance



PT 34: Species identification (voluntary)

Content of sample (vial)	*C. jejuni*	*C. coli*	*C. lari*	Not able to identify	No growth	Growth of other
1. *C. jejuni* & *Escherichia coli*	**30**			1		
2. *C. lari*			**31**			1
3. *C. coli*		**31**				
4. *C. coli*		**31**				
5. Negative					**26**	**5**
6. *C. lari*			**31**			
7. *C. coli*		**31**				
8. *Escherichia coli*					**8**	**23**
9. *C. jejuni*	**30**	1				
10. *C. jejuni* & *Escherichia coli*	**30**			1		

PT 35 – detecton and species identification

Proficiency test no. 35

The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in boot sock samples.

- Detection of *Campylobacter* spp. in boot sock samples from chicken houses (animal samples)
- Species identification of *Campylobacter*
- 18 samples: 6 low level, 6 high level, 6 negative
- Recommended method was procedure B (enrichment in Preston broth) in ISO 10272-1:2017, but other methods allowed
- Enough material for using both direct and enrichment procedures (if of interest for the laboratory)

PT 35: Contents and procedure: boot sock samples

- 18 freeze-dried vials (with or without *Campylobacter* and/or other bacteria)
- 18 numbered plastic bags, each containing one boot sock sample, about 20 ml liquid and some solid material from litter and faeces
- Reconstitute each vial in 5 ml BPW and make two tenfold dilutions
- Add 2 ml of the diluted vial to the corresponding boot sock
- Follow the method(s) of choice for
  - detection
  - species identification

} of *Campylobacter* spp.

Description of the 18 vials in PT 35

Sample No.	Content in vial	Batch No.	Level	log cfu/vial	log cfu/sock	SD (log/cfu)
11	*C. jejuni*	SVA074	low	4.85	2.45	0.08
12	*E. coli*	SVA079		4.29	1.89	0.06
13	Negative					
14	*C. coli*	SVA075	low	4.46	2.06	0.05
15	*C. lari*	SVA080	high	5.78	3.38	0.08
16	*E. coli*	SVA079		4.29	1.89	0.06
17	*C. lari*	SVA080	high	5.78	3.38	0.08
18	*C. lari*	SVA078	low	4.76	2.36	0.06
19	*E. coli*	SVA079		4.29	1.89	0.06
20	*C. lari*	SVA078	low	4.76	2.36	0.06
21	*C. coli*	SVA076	high	5.28	2.88	0.08
22	*C. jejuni*	SVA073	high	7.12	4.72	0.06
23	Negative					
24	*C. jejuni*	SVA073	high	7.12	4.72	0.06
25	*C. coli*	SVA075	low	4.46	2.06	0.05
26	*C. jejuni*	SVA074	low	4.85	2.45	0.08
27	Negative					
28	*C. coli*	SVA076	high	5.28	2.88	0.08

PT 35: Quality control

- Vials produced and tested for homogeneity and stability by the EURL
- Tests were done on vials with *Campylobacter* in duplicates to test stability during transport conditions both before and after dispatch
- The complete test was performed the day after dispatch in best case conditions

Test of stability during transport conditions

Test occasion	Storage conditions	Procedure	No. of samples tested
Before dispatch	Best case	A + B + VC	Each vial with *Campylobacter* × 2
Before dispatch	Worst case	A + B + VC	Each vial with Campylobacter × 2
Just after dispatch	Best case	A + B	The complete test
1 week after dispatch	Worst case	A + B + VC	Each vial with *Campylobacter* × 2

Best case: 5 °C for 24 h

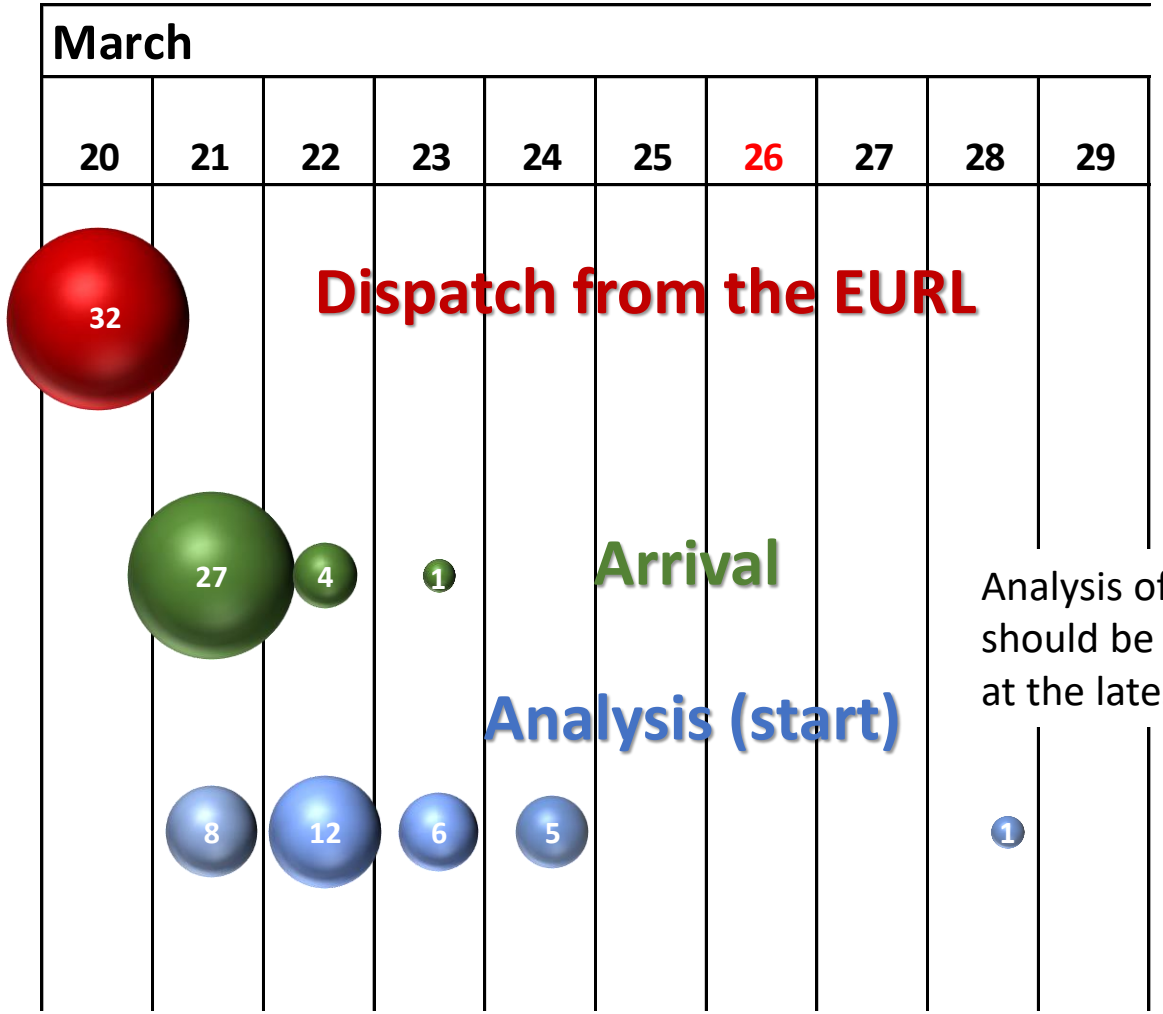
Worst case: 5 °C for 24 h, 15 °C for 24 h, and 5 °C for 24 h

A Bolton, **B** Preston, **VC** Viable count

PT 35: Preparation of the boot sock samples

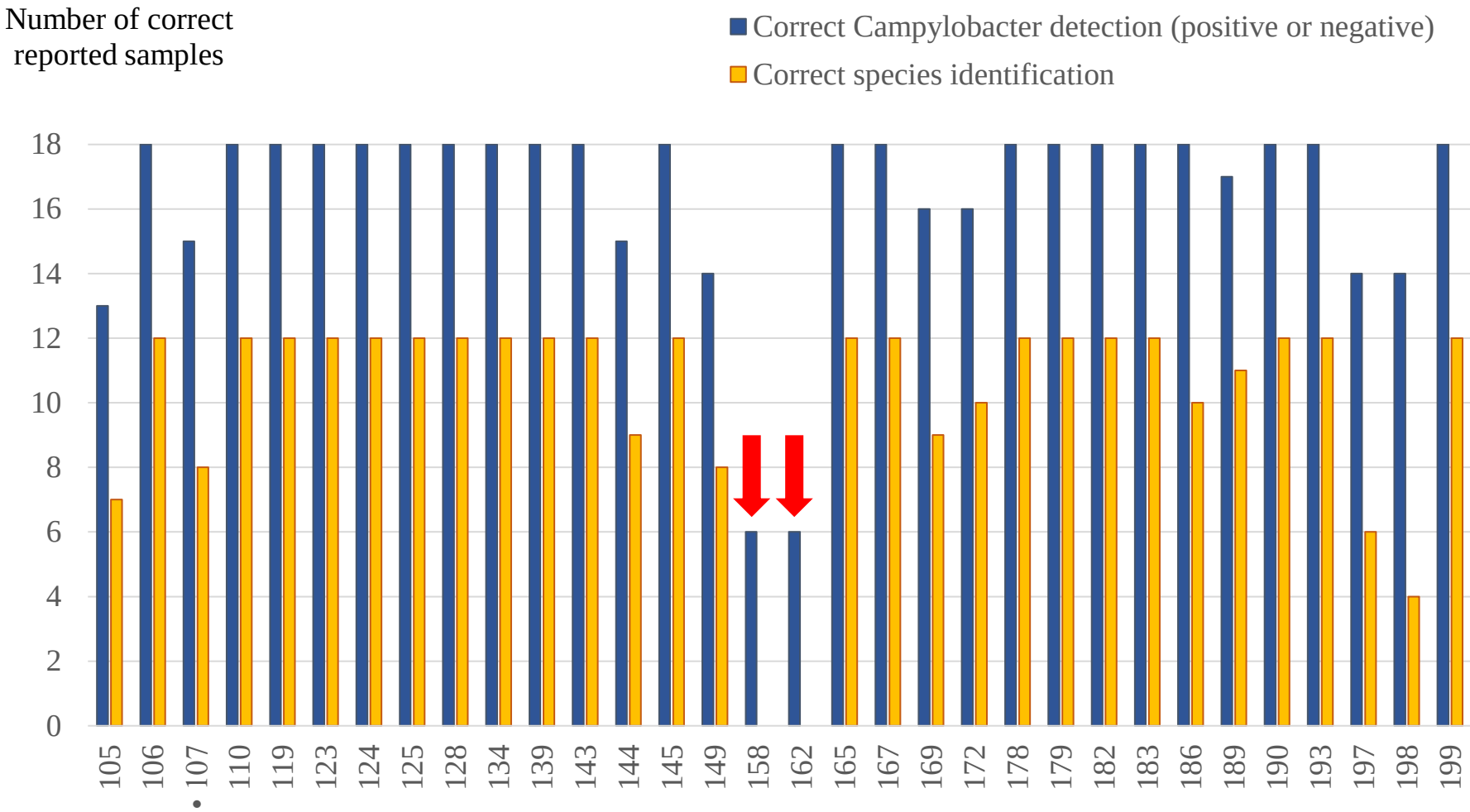
- Freezed *Campylobacter*-free caeca thawed, cut and placed in a stomacher bag and mixed with BPW
- Suspensions pooled into batches and mixed with Cary Blair transport medium to a suitable consistency
- 20 ml of the suspension and some litter material added to a plastic bag with a boot sock, one for each sample
- Samples of a specific number made from the same batch of caecal suspension
- The sock samples stored at 4 °C over the weekend

PT 35: Time to arrival & start of analysis

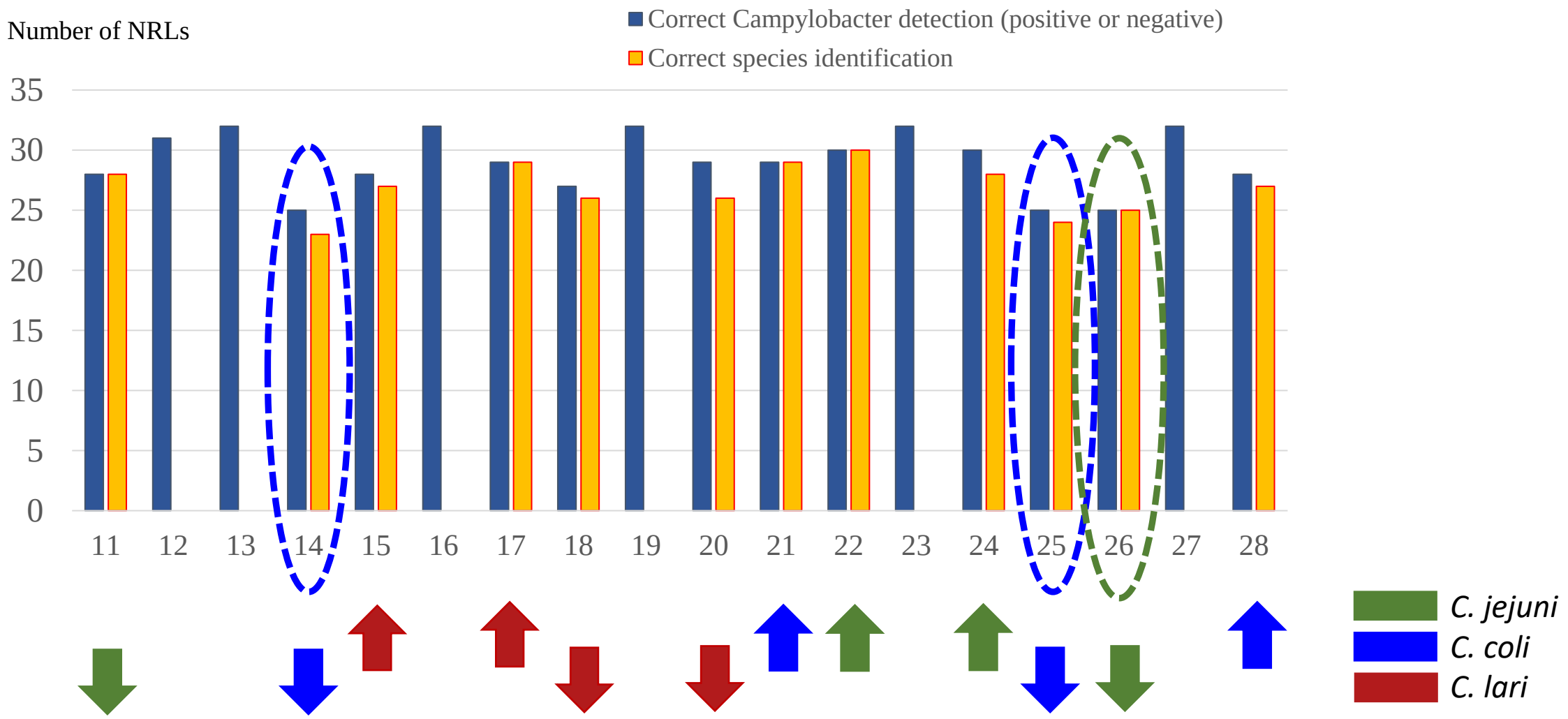


Analysis of the samples included in PT 35 should be started as soon as possible and at the latest on the **24th of March 2023**.

PT 35: Correct reported results per lab



PT 35: Correct reported results per sample

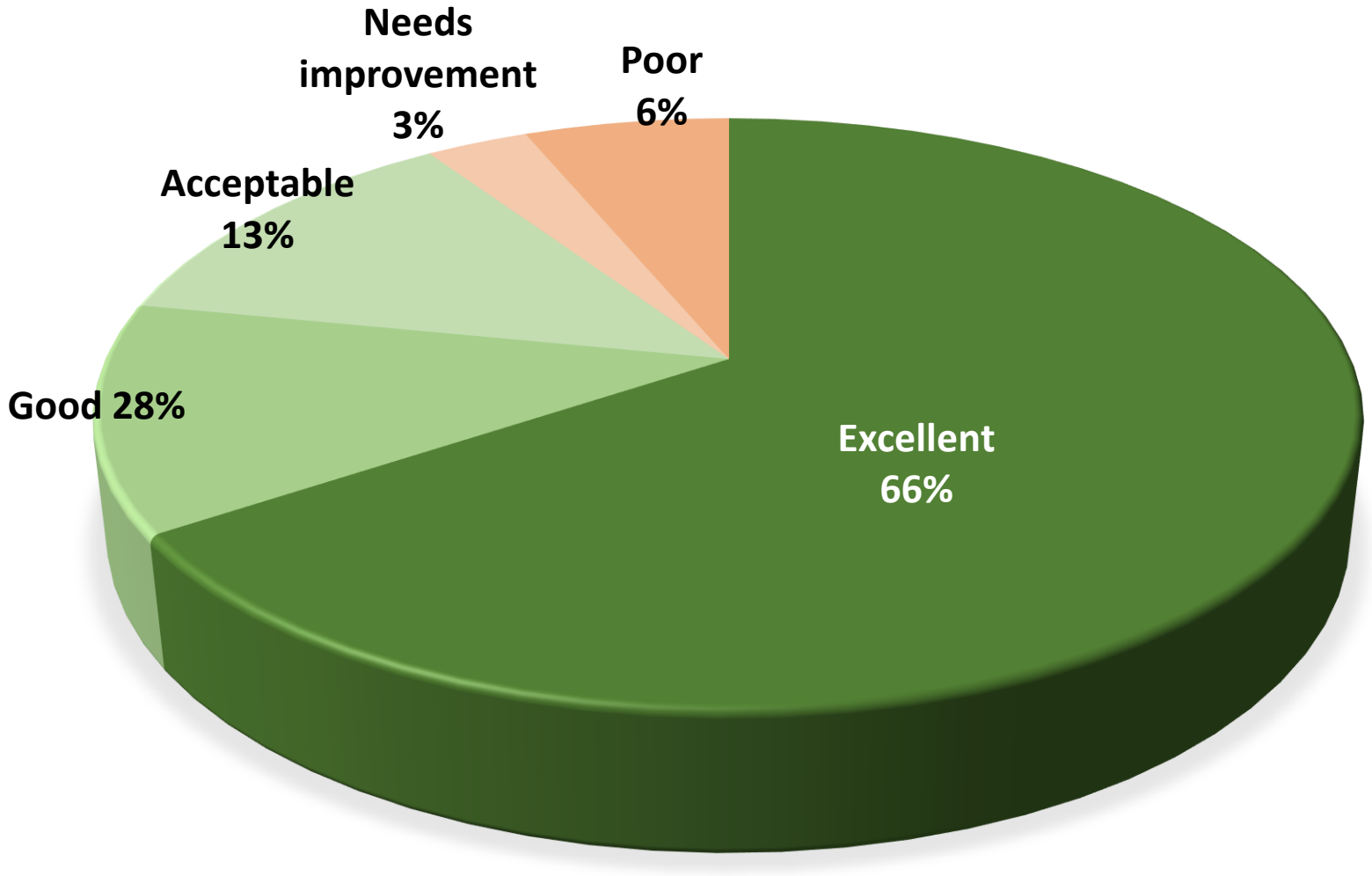


PT 35: Combined Performance grade

*Table showing the **minimum number of correct results** needed for each performance grade*

	Category of samples			Measures on the lower limit of each grade					
Performance grade	**Low level**	**High level**	**Neg**	**Se low**	**Se high**	**Se total**	**Acc**	**Sp**	**Se id**
Excellent	6	6	6	100 %	100 %	100 %	100 %	100 %	95 %
Good	4	5	6	67 %	83 %	75 %	83 %	100 %	85 %
Acceptable	3	4	5	50 %	67 %	58 %	67 %	83 %	70 %
Needs improvement	2	3	4	33 %	50 %	42 %	50 %	67 %	57 %

PT 35: Overall performance in detection of *Campylobacter*



PT 35: Species identification

- 7 misidentifications (and 4 cases of not able to identify species)
  - 3 cases: Sample No. 20 (*C. lari*) reported as *C. jejuni*
  - 2 cases: Sample No. 24 (*C. jejuni*) reported as *C. lari*
  - 1 case: Sample No. 14 (*C. coli*) reported as *C. jejuni*
  - 1 case: Sample No. 24 (*C. lari*) reported as *C. coli*
- Performance in identification: 1 NRL below acceptable limit

Thank you for listening!